On the electrical double layer contribution to the interfacial tension of protein crystals

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We study the electrical double layer at the interface between a protein crystal and a salt solution or a dilute solution of protein, and estimate the double layer's contribution to the interfacial tension of this interface. This contribution is negative and decreases in magnitude with increasing salt concentration. We also consider briefly the interaction between a pair of protein surfaces.

I. INTRODUCTION

Protein crystallisation is not only of immense practical importance, but also attracts interest as a phenomenon in its own right. The conditions under which crystallisation readily occurs are elusive and vary unpredictably, and a number of hypotheses to explain this have been put forward [1, 2, 3, 4, 5, 6]. The process of crystallisation, as with other first-order phase transitions, begins with nucleation, where a microscopic nucleus of the crystalline phase first forms. The elementary theory of nucleation, generally called classical nucleation theory [7], predicts a rate of nucleation which varies as the exponential of minus the cube of the interfacial tension and hence is very sensitive to the magnitude of the interfacial tension. In the present paper, we extend earlier work of one of us on the effect of salt on the bulk phase behaviour [8], to calculate the effect of salt on the interfacial tension and electrical structure at the surface of a protein crystal. Our calculation can be compared to the recent work by Haas and Drenth who also develop a theory for the interfacial tension of protein crystals [9]. Their theory is of the Cahn-Hilliard type and does not treat electrostatic effects explicitly.

It is a general consequence of the long range nature of the Coulomb law that all bulk phases have to be electrically neutral and space charge effects are confined to interfacial regions. The consequences of charge neutrality for membrane equilibrium were explored by Frederick Donnan as long ago as 1911 [10]. Much more recently, the electrical structure of the interface in an electrolyte near the critical point was studied by Nabutovskii and Nemov [11]. In a crystal of charged protein molecules, the counterion concentration in the interior of the crystal will be higher than in the salt solution outside the crystal and the coion concentration will be lower inside the crystal. The higher counterion concentration and lower coion concentration will be such that the net charge density in the crystal vanishes. If one can ignore specific ion interactions and the effects of non-ideality, the ion concentrations can be determined using Donnan's 1911 theory, treating the protein-solution interface as a membrane. This was done in Ref. [8].

In the vicinity of the interface, the ion densities must vary smoothly between the interior of the crystal and the exterior salt solution. In particular, as shown in Fig. 1, one would expect counterions to spill out into the salt solution and coions to similarly spill into the protein, leading to the formation of an electrical double layer across the interface. The space charge gives rise to an electric field and a jump in the mean electrostatic potential between the salt solution and the protein crystal. This potential difference is sometimes termed the Donnan potential, or the Galvani potential [12]. In Donnan's theory, this potential jump is self-consistently responsible for the change in ion concentrations in the bulk phases.

In the theory of metals, a closely analogous phenomenon occurs at a metal surface where the electron density spills out into vacuum. The resulting double layer often makes an important contribution to the work function of metals, and was one of the early targets for density functional theory [13]. In the metal case there is only one density, namely the electron density, which vanishes in the vacuum. The electron density in the interior is very high and the electrons form a highly degenerate Fermi liquid, so that careful attention has to be paid to exchange and correlation effects. In contrast, in the protein case one has two densities, for counterions and coions, which approach a common value in the salt solution outside the crystal. Moreover, for a 1:1 electrolyte in a high dielectric solvent, correlation effects are not that important and for simplicity in our calculation we ignore them.

In the next section we determine the potential profile at the crystal-solution interface, and calculate the associated free energy which represents the contribution of the electrical structure to the interfacial tension. The third section contains results of these calculations along with approximate analytic expressions for the magnitude of this contribution. The last section is a conclusion.

II. CALCULATION

We consider the case where we have a dense protein crystal coexisting with a very dilute protein solution, sufficiently dilute that the few protein molecules in it can be neglected and it treated as simply a salt solution. Rather than considering a specific protein crystal, we take over the jellium concept from the theory of metals to make a general estimate of the effect of salt on the interfacial tension. We replace the detailed charge density due to the
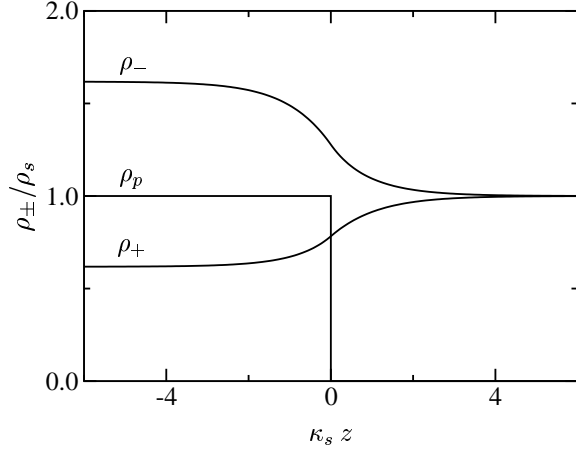
FIG. 1: Ion density profiles for jellium model of a protein crystal-solution interface. The density profiles are computed from the numerical solution to the Poisson-Boltzmann equation, and have been normalised by the salt concentration ρ_s. In this example, the protein charge density ρ_p (assumed positive) was chosen equal to ρ_s. The densities of counter and coions are ρ_- and ρ_+, respectively. Distance is normalised by the Debye screening length defined in Eq. (5).

protein molecules by a uniform background charge density, cut off abruptly at the interface. Thus our model of the bare protein crystal comprises a uniformly charged half-space, with a charge density

$$\rho_{\text{back}}(z) = \begin{cases} \rho_p & (z < 0) \\ 0 & (z > 0) \end{cases} \tag{1}$$

where ρ_p is the mean charge density in the protein crystal. For definiteness we take the protein to be positively charged so the counterions are negative and the coions are positive; both are monovalent. The discontinuity in background charge density at $z = 0$ is dressed by the counterion and coion densities $\rho_-(z)$ and $\rho_+(z)$. These satisfy $\rho_\pm \to \rho_s$ as $z \to \infty$ where ρ_s is the salt concentration, and $(\rho_- - \rho_+) \to \rho_p$ as $z \to -\infty$.

For notational convenience, we work in units where $e = k_BT = 1$. In these units, the Coulomb potential energy U between a pair of elementary charges separated by r is $U = l_B/r$, where l_B is the Bjerrum length, equal to $0.72\,\text{nm}$ in water at room temperature ($l_B = e^2/4\pi\epsilon k_BT$). We assume a constant value of l_B, and ignore dielectric effects.

Below, we will find that in the limit of very large salt concentrations the potential is uniform everywhere, in the protein crystal and in solution, and so can be set equal to zero. In this limit the only relevant contributions to the free energy are independent of salt concentration. For the purposes of considering the effect of reducing the salt concentration, we assume that when the concentration is reduced the protein crystal remains unchanged and interacts with the salt ions solely through a mean electrostatic potential, which we will calculate. We can

then approximate the protein crystal by a uniform background charge density, Eq. (1), and ignore the rest of the protein interactions until we come to estimate their contribution to the interfacial tension. These assumptions are reasonable if the interactions of the protein molecules are strong enough to form a dense, relatively rigid, crystal.

We will use a grand potential, Ω_{el}, which contains only ideal solution terms for the ions and the associated electric field at the interface. We use a grand potential because as usual the calculation of the interfacial tension is easiest at fixed chemical potential not density. The grand potential Ω_{el}, is,

$$\Omega_{el} = \int_{-\infty}^{\infty} dz\, \omega(z), \quad \omega = \sum_{i=\pm} \rho_i(\log\frac{\rho_i}{\rho_s} - 1) + \frac{E^2}{8\pi l_B}. \tag{2}$$

The first terms in ω are the ideal solution terms (the ions share a common chemical potential $\mu_\pm = \log\rho_s$). The last term is the electrostatic energy, wherein $E = -d\phi/dz$ is the electric field strength corresponding to an electrostatic potential ϕ which satisfies the Poisson equation,

$$\frac{d^2\phi}{dz^2} + 4\pi l_B(\rho_+ - \rho_- + \rho_{\text{back}}) = 0. \tag{3}$$

A correlation term $\Omega_{\text{corr}}[\rho_\pm(z)]$ could also be included in Eq. (2) but, as already mentioned, for the purposes of the present calculation we omit correlation effects and work within the simple mean field theory.

The variational principle $\delta\Omega_{el}/\delta[\rho_\pm(z)] = 0$ applied in this problem yields $\rho_\pm(z) = \rho_s \exp[\mp\phi]$. The electrostatic potential then satisfies the Poisson-Boltzmann equation,

$$\frac{d^2\phi}{dz^2} - \kappa_s^2 \sinh\phi = \begin{cases} -4\pi l_B\rho_p & (z < 0) \\ 0 & (z > 0) \end{cases} \tag{4}$$

where

$$\kappa_s^2 = 8\pi l_B\rho_s. \tag{5}$$

Equation (4) can be integrated once with respect to ϕ to obtain

$$\left(\frac{d\phi}{dz}\right)^2 = \begin{cases} 2\kappa_s^2\,(\cosh\phi - \cosh\phi_D) \\ \qquad -8\pi l_B\rho_p\,(\phi - \phi_D) & (z < 0) \\ 2\kappa_s^2\,(\cosh\phi - 1) & (z > 0) \end{cases} \tag{6}$$

where ϕ_D is the Donnan potential, the potential ϕ in the bulk of the protein crystal, so $\phi \to \phi_D$ as $z \to -\infty$. The potential in the bulk of the solution is 0, $\phi \to 0$ as $z \to \infty$.

For $z > 0$ Eq. (6) can be integrated analytically [14] to get $\phi = 2\log[(1 + Ce^{-\kappa_s z})/(1 - Ce^{-\kappa_s z})]$ where $C = \tanh(\phi_0/4)$ and $\phi_0 = \phi(0)$ is the value of the potential at the interface itself. For $z < 0$, it is not possible to integrate analytically, it must be done numerically. Now,
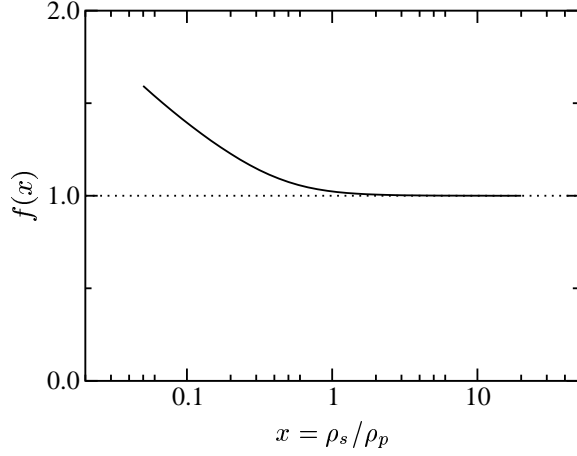
FIG. 2: Numerical factor in the interfacial tension, Eq. (17), as determined from the full numerical solution to the Poisson-Boltzmann equation.

the charge densities for the co- and counterions in the bulk of the protein crystal (i.e., $z \to -\infty$) are

$$\rho_{\pm}(-\infty) = \rho_s \exp(\mp\phi_{\mathrm{D}}). \qquad (7)$$

Charge neutrality imposes

$$\rho_+(-\infty) + \rho_p = \rho_-(-\infty). \qquad (8)$$

Combining these 2 equations yields an equation for the Donnan potential

$$\sinh\phi_{\mathrm{D}} = \frac{\rho_p}{2\rho_s}. \qquad (9)$$

and using this equation and Eq. (5) in Eq. (6) we have

$$(d\phi/dz)^2 = 2\kappa_s^2[\cosh\phi - \cosh\phi_{\mathrm{D}} \\ -(\phi - \phi_{\mathrm{D}})\sinh\phi_{\mathrm{D}}] \quad (z < 0). \quad (10)$$

Matching $d\phi/dz$ across $z = 0$ using Eq. (6) for $z > 0$ and Eq. (10) for $z < 0$, gives

$$\phi_0 = \phi_{\mathrm{D}} + (1 - \cosh\phi_{\mathrm{D}})/\sinh\phi_{\mathrm{D}}. \qquad (11)$$

This not only fixes the complete solution for $z > 0$, but can also be used as the starting point for a numerical integration of Eq. (10) into the $z < 0$ half-space. Note that if Eq. (10) is used to determine $d\phi/dz$, the negative root should be taken; also the term $[\ldots]$ in this equation has a geometric interpretation as the distance between $\cosh\phi$ and its tangent at $\phi = \phi_{\mathrm{D}}$, and thus is always positive.

The benefit of starting with an expression for the grand potential is that the electrical contribution to the interfacial tension, $\Delta\gamma_{\mathrm{el}}$, is easily calculated. The interfacial tension is the difference between the actual grand potential per unit area of the interface and that it would have if each of the 2 phases continued unperturbed right up to

a sharp dividing line between them. We therefore have to calculate the grand potential then subtract the grand potentials for the bulk protein and solution states, thus

$$\Delta\gamma_{\mathrm{el}} = \int_{-\infty}^{\infty} dz\,[\omega(z) - \theta(-z)\omega(-\infty) - \theta(z)\omega(+\infty)] \quad (12)$$

where $\theta(z)$ is the Heaviside step-function, and $\omega(\pm\infty)$ are the limiting values of the grand potential density at $z \to \pm\infty$. They may be obtained from the definition of ω in Eq. (2) and Eq. (7) for the crystal,

$$\begin{aligned} \omega(-\infty) &= -2\rho_s\,(\cosh\phi_{\mathrm{D}} - \phi_{\mathrm{D}}\sinh\phi_{\mathrm{D}}) \\ \omega(+\infty) &= -2\rho_s \end{aligned} \qquad (13)$$

noting that in the bulk phases the electric field is zero. Now, using Eq. (2) together with $\rho_{\pm} = \rho_s\exp(\mp\phi)$ and $E(z) = -d\phi/dz$ from Eqs. (6) and (10), we can write $\omega(z)$ as a function of ϕ. Using this together with Eq. (13) we can then write Eq. (12) as

$$\Delta\gamma_{\mathrm{el}} = 2\rho_s \int_{-\infty}^{\infty} dz\,\phi[\sinh\phi - \theta(-z)\sinh\phi_{\mathrm{D}}]. \qquad (14)$$

III. RESULTS

Before turning to the results of our full calculations, we discuss the high salt limit. If $\rho_s \gg \rho_p$ then $\phi \ll 1$ everywhere and the Poisson-Boltzmann equation (Eq. (6) for $z > 0$ and Eq. (10) for $z < 0$) can be linearised. The Donnan potential is $\phi_{\mathrm{D}} = \rho_p/2\rho_s$, the potential at $z = 0$, Eq. (11), becomes $\phi_0 = \phi_{\mathrm{D}}/2$, and the solution to the linearised Poisson-Boltzmann equation is

$$\phi = \begin{cases} (\phi_{\mathrm{D}}/2)\,[2 - \exp(\kappa_s z)] & (z < 0) \\ (\phi_{\mathrm{D}}/2)\exp(-\kappa_s z) & (z > 0) \end{cases}. \qquad (15)$$

In Eq. (14) these yield for the interfacial tension

$$\Delta\gamma_{\mathrm{el}} = -\frac{\rho_s\phi_{\mathrm{D}}^2}{2\kappa_s} \quad (\rho_s \gg \rho_p). \qquad (16)$$

This already shows the main features of the full solution, being *negative* and of decreasing magnitude as the salt concentration increases. The fact that the result should be negative can be seen directly from Eq. (12). If the ion density profiles were such that they remained constant right up to the interface where they had a sharp jump between the bulk values, then Eq. (12) would give $\Delta\gamma_{\mathrm{el}} = 0$. The fact that the ion density profiles are relaxed compared to such a trial density profile implies that $\Delta\gamma_{\mathrm{el}} < 0$. Note that the electrostatic energy is always positive though (it is the last term in Eq. (2)).

For the general case, we note that κ_s^{-1} is the only length scale that enters into the Poisson-Boltzmann equation, thus dimensional analysis allows us to write

$$\Delta\gamma_{\mathrm{el}} = -\frac{\rho_s\phi_{\mathrm{D}}^2}{2\kappa_s}\,f(\frac{\rho_s}{\rho_p}) \qquad (17)$$
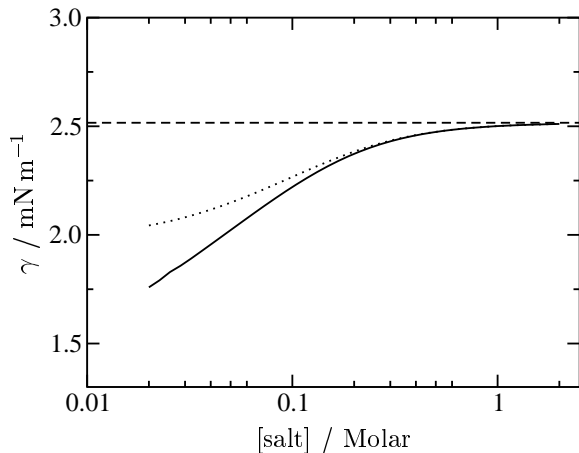
FIG. 3: Predicted interfacial tension for lysozyme crystals, based on the present work and previous models [3, 8]. The dashed line is the high-salt limit γ_0 estimated in the text. The solid line incorporates the correction in Fig. 2. The dotted line is the result if $f = 1$ is used in Eq. (17).

where $f(x)$ is a numerical factor which amends the high salt scaling limit and depends only on the ratio $x = \rho_s/\rho_p$. We have implemented a numerical scheme to integrate Eqs. (10) and (14), and determine $f(x)$. The result is shown in Fig. 2. The function obviously obeys $f \to 1$ as $\rho_s/\rho_p \to \infty$, but the interesting thing is that f does not deviate greatly from unity even at much lower salt concentrations, for example $f \approx 1.4$ at $\rho_s/\rho_p \approx 0.1$. This indicates that the high-salt scaling limit Eq. (16) is not a bad approximation even at much lower salt concentrations, provided that we use the exact expression for the Donnan potential given in Eq. (9).

We can further approximate the contribution to the surface potential by using the high salt expression for ϕ_D, which is obtained by linearising Eq. (9), and is $\phi_D = \rho_p/(2\rho_s)$. With this approximation we obtain $\Delta\gamma_{el} = -\rho_p^2/(8\rho_s\kappa_s)$. At high salt the contribution of the ions to the thermodynamic potential of the protein crystal is again obtained by linearisation and is given by $\omega(-\infty) = -2\rho_s + \rho_p^2/(4\rho_s)$. The first term is simply $\omega(+\infty)$, it is linear in ρ_s and has no effect, the second term is proportional to the square of the protein density and so is a, positive, contribution to the second virial coefficient. This has been noted before, see Refs. [8, 15]. So, the contribution to the interfacial tension is of order of the contribution to the free energy density times the Debye length κ_s^{-1}, which defines the thickness of the interface of course. This is what we might have expected simply on the basis of dimensional analysis, although dimensional analysis would not of course have told us that $\Delta\gamma_{el}$ is negative.

Our proteins are charged colloids and at least at high salt, charged colloids are often viewed as repelling each other via a Derjaguin-Landau-Verwey-Overbeek (DLVO) pairwise repulsion which has the form $Q^2 l_B \exp(-\kappa_s r)/r$

for a pair of colloids each with charge Q and r apart [14]. Treating such repulsions within a mean-field approximation yields a contribution to the free energy density which is exactly what we have found, $\rho_p^2/(4\rho_s)$ [16]. This free energy density is just a free energy per protein molecule of order $Q\rho_p/(4\rho_s)$. If we assume an interface of width κ_s^{-1} at the edge of a crystal, within which there are $(\rho_p/Q)\kappa_s^{-1}$ protein molecules per unit area of the interface, each of which has a DLVO energy which is some fraction of its value in the bulk, then the contribution of the DLVO repulsion to the interfacial tension is of order $-\rho_p^2/(\rho_s\kappa_s)$. Thus in the high salt limit our results for the contributions to the bulk free energy density and to the interfacial tension are of the same order as those obtained by assuming the protein molecules repel each other via a pairwise repulsion of the DLVO form; provided we use a mean-field approximation for the bulk and assume a protein free energy density profile of width κ_s^{-1} at the interface. Note that if we used a DLVO potential and assumed its contribution to $\Delta\gamma_{el}$ was simply due to a missing nearest-neighbour repulsion per molecule at the surface, then $\Delta\gamma_{el} = Q^2 l_B \exp(-\kappa_s b)/b^3$, where b is the lattice spacing. This is rather different from our Donnan-potential result. Our finding that, in the high salt limit, our results obtained via a Donnan potential approach plus the Poisson-Boltzmann equation for the interface profile, can be obtained (up to numerical prefactors) via a DLVO potential is consistent with work on charged synthetic colloids. This work found that a DLVO pair potential is adequate to describe the system, see for example Ref. [17]. By contrast, at low salt, charged colloids are not well described by a pair potential, see Refs. [18, 19] and references therein.

A. Predictions for lysozyme

To make these results more concrete, we now consider a specific example. We choose the lysozyme/NaCl/water system, since considerable information has been collected here. Elsewhere it has been shown that the crystallisation boundary in this system and the second virial coefficient data can be fitted reasonably well with a model of hard spheres with sticky patches [8, 20]. The sticky patches are short-ranged directional potential wells. Following Ref. [8] we perform calculations for a well depth $\epsilon = 7.4\,k_BT$ per patch. With attractions of this strength, and at high salt concentration, the protein crystal coexists with a very dilute protein solution. We estimate the interfacial tension in this model, in the high salt limit, to be $\gamma_0 \approx \epsilon/l^2$ where l^2 is the interfacial area per protein [3]. Since the dimensions of lysozyme are $4.5 \times 3.0 \times 3.0\,\text{nm}^3 \approx l^3$, we estimate $l^2 \approx 12\,\text{nm}^2$ and consequently $\gamma_0 \approx 0.6\,k_BT\,\text{nm}^{-2} = 2.5\,\text{mN m}^{-1}$ (by way of comparison, this is intermediate between the interfacial tension of a clean oil/water interface typically $50\,\text{mN m}^{-1}$, and interfacial tensions that have been measured between colloidal phases in the range 1–$20\,\mu\text{N m}^{-1}$

[21, 22]). To use our theory for the electrical correction to γ_0, we need the charge density in the crystal. Lysozyme has quite a high, pH-dependent charge, Q, and for the purpose of the present calculation we use $Q = 10$ [23] which gives $\rho_p \approx Q/l^3 \approx 0.25 \, \text{nm}^{-3} = 0.4 \, \text{M}$.

Fig. 3 shows the predicted surface tension, $\gamma = \gamma_0 + \Delta\gamma_{\text{el}}$, as a function of salt concentration. As might be expected, the electrical structure at the protein crystal-solution interface starts to significantly reduce the interfacial tension for $\rho_s \lesssim 0.4 \, \text{M}$. As the physiological salt concentration is $0.15 \, \text{M}$, the interfacial tension of a protein crystal *in vivo* is significantly reduced by the double layer. Our assumption that the density of protein molecules in the solution phase coexisting with the crystal is so low that it can be approximated by zero, is reasonable down to a salt concentration of about $0.1 \, \text{M}$, with sticky patches of strength $\epsilon \approx 7.4 \, k_{\text{B}}T$ [8]. Below this we would need to take account of the density of protein in the solution phase coexisting with the crystal.

We have been unable to find any experimental determination of the protein crystal-solution interfacial tension, but we hope that our calculations may stimulate experimental work to confirm our results. However, a prediction can be extracted from our theory for the variation in protein solubility, i.e., the lysozyme concentration in the fluid phase coexisting with the crystal, as a function of the concentration of NaCl [15, 24]. As noted above, at high salt concentration the grand potential density in the protein crystal is $\omega(-\infty) = -2\rho_s + \rho_p^2/(4\rho_s)$. This implies a contribution to the excess chemical potential of a protein molecule of $Q\rho_p/(2\rho_s)$. Treating the solution phase which coexists with the crystal as ideal (since the protein concentration is very small) this variation of the excess chemical potential with salt concentration yields for the solubility as a function of salt concentration

$$\ln\rho_{\text{sol}}(\rho_s) = \ln\rho_{\text{sol}}(\rho_s \to \infty) + \frac{Q\rho_p}{2\rho_s}, \qquad (18)$$

where $\rho_{\text{sol}}(\rho_s)$ is the charge density of the protein molecules in the dilute solution which coexists at equilibrium with the protein crystal. This recovers a result also found for the high salt limit of the theory in Ref. [8]. As the first term on the right-hand side is a constant, our prediction is that the logarithm of the solubility is inversely proportional to the salt concentration. This is same dependence as found in experiment [24] but, as noted in Ref. [8], the slope predicted by Eq. (18) is close to twice the slope measured for the experimental data.

B. Comparison with surface-force apparatus measurements

A closely related problem to that of the free energy of a single crystal surface is that of the potential of mean force per unit area $W(s)$ between a pair of protein surfaces as their separation s is varied. This is related to the interfacial tension problem since $W(0) = -2\gamma$ if we choose

the reference state such that $W(\infty) = 0$. Its derivative with respect to separation is the negative of the force per unit area between two surfaces. We expect that the important contribution to W from the sticky patches will be operative only when $s < \delta$, where δ is the range of the sticky patch potential. Previous work on the phase diagram suggests δ is only a few percent of the protein diameter [8, 20]. If $s > \delta$, W arises from the overlap of the double layers and should be repulsive since both surfaces carry the same charge. In fact, we would expect W to exhibit a potential barrier at $s \approx \delta$, of height $-2\Delta\gamma_{\text{el}}$ and extending out to a distance of order κ_s^{-1}.

The interaction between protein surfaces is more experimentally accessible than the interfacial tension, as it can measured via a direct force experiment. Sivasankar *et al* have recently looked at the interaction between streptavidin-covered surfaces using a surface force apparatus [25]. This is not quite our system as we consider a bulk crystal whereas the experiments are on streptavidin monolayers, but so long as the Debye screening length is smaller than the protein diameter, the thickness of the layer over which the ion densities vary will not be much larger than the thickness of a monolayer of protein molecules. Sivasankar *et al* found that the interaction was well described by the non-linear Poisson-Boltzmann equation with a fixed effective surface charge density of order $10 \, \text{mC} \, \text{m}^{-2}$. Whilst the present theory does not calculate the interaction between a pair of surfaces, we note that the electrostatic potential outside the crystal is the same as would be obtained for a surface charge density $\sigma = (4\rho_s/\kappa_s)\sinh(\phi_0/2)$, where ϕ_0 is the potential at the surface determined earlier (the simplest way to derive this result is via matching the electric field strength at $z = 0$). For example, for lysozyme at $\rho_s = \rho_p = 0.4 \, \text{M}$, we find, using this equation, an effective surface charge density $\sigma \approx 0.06e \, \text{nm}^{-2} \approx 10 \, \text{mC} \, \text{m}^{-2}$, of exactly the same magnitude seen by Sivasankar *et al* [25].

Finally we comment briefly on the case of a single protein molecule approaching the surface of a protein crystal. In this case one would expect a barrier of height $\approx Q\phi_0$ to be present in the potential of mean force, before the isolated protein encounters the short range attraction.

IV. CONCLUSION

We have estimated the contribution to the interfacial tension of a protein crystal made by the electrical double layer at the interface. We have used a jellium model in which the protein is replaced by a uniform background charge density, and have solved the Poisson-Boltzmann equation for the ion density profiles for a jellium half-space. Whilst a full solution cannot be obtained analytically, we find a good approximation is to use the high-salt limit result in Eq. (16) with the Donnan potential given in Eq. (9). The contribution of the double layer is, perhaps surprisingly, *negative* and for a protein not too close to its isoelectric point and of a typical size, it

amounts to roughly 10% of the total interfacial tension at salt concentrations of order 0.1 M, see Fig. 3. As the salt concentration is increased the contribution of the double layer *decreases*, due to the decreasing step in potential as the interface is crossed and its decreasing width. We have also briefly considered the potential of mean force between a pair of protein surfaces as they are brought together. The mean force is repulsive and agrees with that found by Sivasankar *et al* [25] for the repulsion between monolayers of the protein streptavidin.

The fact that the contribution of the double layer to the interfacial tension is negative and largest at low salt concentrations suggests that proteins will crystallise more readily at low rather than at high concentrations of a salt such as NaCl. At lower salt concentrations the interfacial tension will be lower, all other things being equal, and the rate of nucleation varies as the exponential of minus the cube of the interfacial tension. However, decreasing the concentration of salt will make the protein crystal more soluble, it stabilises the solution phase at the expense of the crystal [8]. Thus if the salt concentration is too low the solution will be the equilibrium phase and crystallisation will not then occur at all. Because lowering the salt concentration lowers both the relative stability of the crystal with respect to the solution and the interfacial tension, we conclude that there is no clear 'best' salt concentration at which to attempt to crystallise a protein. Also, note that by salt we mean a salt such as NaCl in the lysozyme system, in which there are no specific ion effects such as binding of an ion to the protein.

As well as the interaction between a pair of protein surfaces, we mention some other directions in which our theory could be extended. Firstly, ion correlations and the effects of an inhomogeneous charge distribution could be included. Secondly, we have neglected excluded volume effects and changes in dielectric properties which are potentially important in the interior of the protein crystal. Thirdly, we have only considered the case where the protein crystal coexists with a dilute protein solution, such that the protein concentration in the solution makes a negligible contribution to the charge balance. It has been suggested that protein crystallisation is facilitated by a metastable liquid-liquid demixing transition [1, 2, 5], in which case one should certainly examine the effect of a more concentrated protein solution phase [9].

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